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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)

In the first year, this program has supported two graduate students (one from EE Department and one from Biochemistry Department) and a postdoctoral fellow (Radiology Department). They have been introduced to the Biomedical NMR Laboratory and the Howard University Cancer Center. The trainees learned the theory and instrumentation of Nuclear Magnetic Resonance imaging and spectroscopy. The students have also rotated through the clinical services in the hospital to learn the mammography procedures. They have participated in the seminar series in the Cancer Center and throughout the campus. The trainees have been introduced the ongoing research projects in the lab. They all have started their research projects with the PI. Based on the preliminary findings, two papers and two posters have been presented in the University Research Forums and in the National Scientific Meetings. From these initial research results and contacts with scientists at Georgetown University, a collaborative partnership grant has been developed, submitted and funded by USAMRMC.

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TABLE OF CONTENTS

| | |
|-------------------------------|----------|
| I. Front Cover | 1 |
| II. SF298 Form | 2 |
| III. Table of Contents | 3 |
| IV. Reports | 4 |
| V. Reportable Outcomes | 7 |
| VI. Appendix | 7 |

IV. Reports

There are two Ph.D. graduate students (Mr. Emmanuel Agwu and Ms. Lisa Kinnard) and one postdoctoral research associate (Dr. Jianwei Zhou) supported by this grant. Mr. Agwu is a 5th yr MD/PhD student pursuing his Ph.D. degree in Biochemistry. Ms. Kinnard is a graduate student from the Department of Electrical Engineering. Dr. Zhou is a chemist in the Department of Radiology. The reports and accomplishments for each individual are listed separately according to the Statement-of-Work in the proposal. The individual reports are followed by a summary of the reportable outcomes including presentations/publications, awards and grant.

1. By Lisa Kinnard, Howard University, Department of Electrical Engineering, Ph.D. student

- **Introduction to the Biomedical NMR Laboratory and Cancer Center**

During the first several months, Dr. Paul Wang introduced me to the Biomedical NMR Laboratory. At this time, Dr. Wang explained the technical capabilities of the equipment as well as the group's current research projects. At this time I also met with mentors to learn the current status of breast cancer research and to determine what possible contributions could be made to the field.

- **Learn NMR instruments**

From the fourth month through the end of the year, I learned the physics and instrumentation of Mammography and MRI. Additionally I learned the about picture archiving communication system (PACS) with regard to its significance in the radiology field and its general impact on hospitals.

- **Seminar Presentation**

In March and April of 2001, I worked on an image processing project and coauthored a poster presentation in the annual meeting in The Annual Pediatric Academic Societies Meetings, May 1-5, 2001, Baltimore, MD.

- **Clinical Preceptorship**

In December of 2000 I began a radiology internship with Dr. Eva Duckett of the Howard University Hospital Radiology department. During this internship, Dr. Duckett trained me in the following areas: 1) Patient management, 2) Screening/Diagnostic procedure, 3) Breast cancer image patterns, 4) Understanding of typical cases versus clinically indeterminate cases, 5) Understanding of geometric distribution (physical locations of tumors), 6) Image patterns of cysts, fibroadenomas, 7) Image pattern analysis of masses vs. microcalcifications and 8) Biopsy procedures. Dr. Matthew Freedman (and various other radiologists) of the Georgetown University Medical Center (GUMC) currently answers any of my questions concerning the mammography images; however, in the future, Dr. Rebecca Zuurbier (director of breast imaging, director of Betty Lou Ourisman Breast Health Center) of the GUMC will be interviewed concerning the screening/diagnostic procedure, patient management, and biopsy procedures of the GUMC.

- **Summary of Accomplishments**

The objective of the research is to develop a system that will act as a consultation system to assist radiologists in determining the likelihood of malignancy. This work can be divided into three parts: determination of image segmentation (separation of tumor from surrounding tissue), calculation of image statistics, and determination of a classification method. During the past year I have completed the following tasks toward the completion of the research:

1. Written report on Mammography which covering:

- a. Physics/Instrumentation of traditional X-ray
- b. Summary of Mammography
 - i. Physics/Instrumentation
 - ii. Film-Screen Combinations
- c. Breast Anatomy/Mammographic Analysis
- d. Clinical Procedures
- e. Summary of image patterns in benign and malignant calcifications in mammography
- f. Summary of image patterns in benign and malignant masses in mammography

2. Performed literature search on image features used for breast cancer diagnosis
3. Programmed statistical equations
4. Digitized mammography films
5. Ran statistics on phantom images
6. Correlated patient records with images in order to locate tumors
7. Surveyed image segmentation techniques
8. Completed first prototype of image segmentation phase

2. By Emmanuel Agwu, an MD/PhD student, PhD candidate in the Biochemistry Department

- **Introduction to the Biomedical NMR Laboratory and Cancer Center**

I was given a tour of the Biomedical NMR Laboratory so that I was able to locate laboratory materials as well as major equipments (e.g. 200 and 400 MHz NMR machines). I was also given a tour of the Cancer Center in order for me to know where other necessary biomedical equipments were located and to meet other scientists conducting research in breast cancer. I was briefed on the on-going projects in our laboratory and give the grant proposal as wells as related journals for thorough review.

- **Learn NMR**

Dr. Wang provided me a week of intensive training on NMR. Aside from many hours of on-hands training, I also enrolled in a NIH graduate course on NMR. Furthermore, I was provided with several textbooks on NMR and MRI theory and applications. During the spring of 2001, I attended courses on MRI theory and application taught by Dr. Wang to the Howard University Radiology residents.

- **Start Biochemical Departmental Course Work**

I am a fifth year MD/PhD student. I have completed the first two years of medical school. I was also enrolled in the Biochemistry Department as a PhD student. I have completed 21 institutional credit courses and passed the qualified examination. I gave a seminar each semester both in the Biochemistry Departmental and the MD/PhD program.

- **Clinical Preceptorship**

As required by the MD/PhD program, I attended a clinical rotation with a clinical preceptor once a week for half a day. This is necessary to keep me abreast on clinical issues while understanding my Ph.D. training.

- **Report to MD/PhD Committee and Biochemistry Department on Research Progress**

The MD/PhD program committee hosts a student research forum every year. In the research forum, the students in the program report on the progress of their research to the rest of the university. I presented a talk on May 17, 2001, entitled "MRS Study of ^{31}P Metabolism in MCF7 Breast Cancer Cells". I also presented a paper in the 12th Annual Research Day in the

Biochemistry Department, entitled "An Improved NMR Perfusion System For Breast Cancer Cell Study". I have submitted and am being accepted to present a paper on October 12, 2001 at the Academic Minority Physicians 15th Annual Scientific Meeting in Washington, DC.

3. By Jianwei Zhou, Ph.D., Research Associate, Department of Radiology, Howard University

• **Introduction of Biomedical NMR Laboratory and Howard University Cancer Center**

In the beginning of February 2000, I joined the Howard University Hospital Cancer Center, as a research associate in the Biomedical NMR Laboratory. At first, I spent one month to learn about the ongoing research projects in this lab. Breast cancer is one of the major research areas of the Cancer Center. The Biomedical NMR Laboratory has been involved in breast cancer research using NMR imaging and spectroscopy techniques since 1989. Our major research interests include a study of metabolism and the responses of perfused breast cancer cells under the drug treatment. I have studied related literature and frequently discussed with Dr. Wang, Dr. Shridhar, and other group members in the lab to appreciate the complexity of the research. As a chemist by training, I have learned the cell culture techniques required for the research such as subculture cells, harvesting cells, how to freeze the cells for storage and to prepare agarose thread containing breast cancer cells for NMR studies. I have learned how to use the shared facilities, such as cold room, incubator, and handling the cells under the hood in the Cancer Center.

• **Participate in weekly Cancer Center seminars**

I participated in many Cancer Center weekly seminars. I also participated in many NMR and cancer related seminars on campus. I attended a workshop entitled "Animal Models in Breast Cancer Imaging" sponsored by the Howard University Cancer Center and Walter Reed Hospital.

• **Learn to use three NMR instruments in the laboratory**

As a NMR spectroscopy chemist, it took me a relatively short time to be familiar with the Varian NMR instruments in the lab. I learned how to obtain good spectra with high signal to noise ratio, how to transfer data between NMR machines and satellite workstations, and data analysis.

• **Take NMR and other courses**

Besides the instrumentation training in the lab, I have learned NMR imaging and spectroscopy theories taught by Dr. Wang. I also took the biochemistry course at NIH. I also learned the cell culture procedures from the Dr. Asafa's lab at the Cancer Center.

• **Start research program**

I participated in the ³¹P NMR study of breast cancer cells including MCF7 wild type (wt), MCF7 drug resistant, MDA231, KB-V-1, and KB-3-1 cells. We presented a poster at the 42nd ENC Conference. Some of the major findings and achievements are listed as follows:

1. The high signal to noise ratio spectra are obtained for all kinds of breast cancer cells.
2. The NMR T1 measurements of phosphorus metabolites for MCF7/wt and MDA231 cells.
3. Constructed a new perfusion system for NMR studies to resolve the bubble problem.
4. Build a gas bubbling system for the cell oxygenation experiments.
5. Start the oxygenation experiment, which is designed to understand the cell oxygenation conditions in our cell perfusion system.
6. Start the drug treatments studies using Doxorubicin.
7. A special spin lock system/technology has been introduced in the cell perfusion NMR experiment.

V. REPORTABLE OUTCOMES

PUBLICATION

1. **Zhou JW, Agwu CE, Li EC, Wang PC.** An Improved NMR Perfusion System For Breast Cancer Cell Study. 42nd Experimental NMR Conference, March 11-16, 2001, Orlando, FL.
2. Ting P, **Wang PC, Kinnard L**, Herman MM, Cohn R. Early EEG and Diffusion MRI (dMRI) Changes in an Experimental Model of Severe Periventricular Leukomalacia (PVL). The Annual Pediatric Academic Societies Meetings, May 1-5, 2001, Baltimore, MD.

AWARDS

1. Mr. Emmanuel Agwu received the Association for Academic Minority Physician, 2001 Minority Medical Student Research Summer Fellowship, a Merck/AAMP scholarship.
2. Mr. Emmanuel Agwu received a 2001 Scandrett Scholarship Award, a scholarship for disable students.

GRANT

Dr. Paul Wang with Dr. Mohamed Chouikha (P.I.) in the Department of Electrical Engineering has submitted a partnership training grant to USAMRMC and it was funded for 2001-2004. This program is a training partnership between Howard University and Georgetown University (Dr. Ben Lo and Dr. Matthew Freedman) to train faculty and students in breast cancer imaging, digital image database library techniques and network communication strategy.

VI. APPENDIX

Appendix 1. The poster from the 42nd Experimental NMR Conference, March 11-16, 2001, Orlando, FL. Entitled “An Improved NMR Perfusion System For Breast Cancer Cell Study”.

Appendix 2. The poster from The Annual Pediatric Academic Societies Meetings, May 1-5, 2001, Baltimore, MD. Entitled “Early EEG and Diffusion MRI (dMRI) Changes in an Experimental Model of Severe Periventricular Leukomalacia (PVL)”.

AN IMPROVED NMR PERfusion SYSTEM FOR BREAST CANCER CELL STUDY

Jianwei Zhou, Chikezirim E. Agwu, Ercheng Li, Paul C. Wang*

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Abstract

In this poster, an improved NMR cell-perfusion system is presented. The perfusion system is driven by a peristaltic pump. The portion of the system before the pump is under negative pressure, while the portion after the pump is under positive pressure. This design helps with the removal of air bubbles from the perfusion medium. Using this perfusion system, a cell viability study of the MCF-7 breast cancer cell line was extended successfully for more than a week. The ³¹P NMR spectrum of the MCF-7 showed three distinct phases: phase 1, 2, and 3. Further characterization of the agarose-enclosed cell perfusion system suggests that the cells utilized aerobic respiration while under perfusion. This was done by perfusing the cells with either 0.1 mM lodoacetamide or 10 mM Pentobarbital. The spectra showed the disappearance of phosphorus metabolites until the eventual cell death. The results clearly demonstrate that the long time bubble-free NMR cell perfusion system could be a useful tool for breast cancer cell research.

Introduction

NMR has been used to monitor the metabolism of cells as well as the responses of cells under drug treatment (1,2,3,4). Usually, the NMR study requires data acquisition over a long period of time, hours or even days. To assure even distribution of nutrients, the cells under study are encased in agarose while being perfused with serum-containing medium (4,5). Over a long acquisition period, the air in the perfusion medium gets released as it comes in contact with the agarose, giving rise to bubble formation. Air bubbles destroy the magnetic homogeneity of the system thus, decreasing the signal-to-noise ratio of the spectrum. This discourages prolonged cell studies. In agarose-enclosed system, it is critical to have an air tight perfusion system to avoid bubble formation inside the NMR tube if a prolonged acquisition period is desired. Here, an improved long time NMR perfusion system is presented. This system releases and traps air bubbles from the perfusion medium prior to the medium reaching the agarose-enclosed cells in the NMR tube.

Experimental Procedure

Sample Preparation: The breast cancer cells, MCF-7, were grown in 10 tissue culture flasks containing MEM growth medium complete medium (10% FBS). The cells (>2x10⁶ cells) were harvested by trypsin in a 15 ml centrifuge tube giving 0.1 ml pellet. The pellet was placed in a 37°C water bath and imbed with 0.7 ml low temperature-gelling agarose solution in PBS (1.1%). The agarose-cell mixture was extroded, under stable pressure, through a 0.5 mm inner diameter plastic tube into an NMR tube containing MEM complete medium. While traversing through the plastic tube, the agarose-cell mixture produces a broad remanaging spaghetti. The NMR tube containing the agarose-enclosed cells was then placed producing a NMR image and perfused at the rate of 0.9 mm/min throughout the acquisition period. Depending on the study, the cells were seeded with MEM complete medium, MEM complete medium containing 0.1 mM lodoacetamide, or MEM complete medium containing 10 mM pentobarbital.

NMR Measurement: All NMR data were collected in Varian VXR-400 NMR instrument at 161.9 MHz for ³¹P with proton decoupling. The temperature of NMR tube inside the magnet was kept at 37°C ± 1°C through the duration of acquisition. The magnetic field lock signal was provided by the D₂ capillaries, which surrounded the outside of RF coil.

An improved NMR cell-perfusion system

According to Henry's law, the mass of a slightly soluble gas that dissolves in a definite mass of a liquid at a given temperature is directly proportional to the partial pressure of that gas,

$$P_1 = K_1 \cdot X_1$$

Where, K_1 is the Henry's law constant, which is a function of temperature. At the equilibrium state, the mole fraction of dissolved gas (X_1) in solution is dependent on the partial pressure of the gas (P_1) on the solution surface and the temperature. For example, at 37°C, X_{O_2} (gas) and X_{CO_2} (gas) are 1.91x10⁻³ (or 10⁻⁴ torr) and 4.07x10⁻⁴ torr, and at 38°C, the X_{O_2} (gas) and X_{CO_2} (gas) are 2.40x10⁻³ torr and 7.51x10⁻⁴ torr, respectively. Since the atmospheric contains -21% of O₂ and -79% of N₂, it follows that about 13.9 ml of gas per liter will be released when the temperature of the solution is changed from 37°C to 38°C at standard atmosphere pressure and equilibrium states. This explains why previously used agarose-enclosed cell perfusion systems frequently produced air bubbles over a prolonged acquisition period.

Fig. 1 shows the improved NMR-perfusion system for breast cancer cells. The agarose-enclosed cells are located in G, parts B, C, including the perfusion tubing A-B, are kept in the thermostatic water bath at 37°C ± 0.1°C. Tube B contains a thread of agarose gel (without cells) which serves as a gas release center. As the cold medium in flask A passes through tubing A-B, it warms up and becomes gas-supersaturated solution. When it flows across the agarose gel thread (gas release center) in tube B, the supersaturated gas is released. Moreover, before the pump (D), the pressure inside the perfusion system is lower than atmospheric pressure (pump pulls medium), and after the pump (D), the pressure inside the system is higher than atmospheric pressure (pump pushes medium). The trapped gas could be removed from the system by an injector through the valves of C or F.

A Prolonged Perfusion System For Breast Cancer Cells

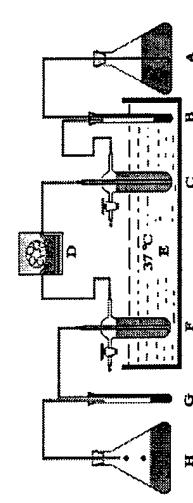


Fig. 1. A) Medium; B) Agarose gel thread without cells; C) First gas trap; D) Peristaltic pump; E) Thermostatic bath; F) Second gas trap; G) Agarose-enclosed cells in NMR tube; H) Waste.

Application Results and Discussion on

Application 1: The baseline experiment of breast cancer cells MCF-7 was obtained over 7 days. Fig. 2 shows a typical ³¹P spectrum of MCF-7 breast cancer cells during perfusion. The metabolic components in Fig. 2 are phosphocholine (PC, 4.10ppm), phosphocholine (PC, 3.30ppm), glycerophosphocholine (GPC, 1.90ppm), glycerophosphocholine (GPC, 0.30ppm), diacylglycerol (DGE, 1.05ppm) and ATP (1.65ppm). Figure 3 shows three kinds of spectra with perfusion time to some individual metabolic components can be identified: Phase 1, 2, and 3. During harvesting and agarose gel encasement, the cells are maintained at a low temperature, which effectively reduce their metabolic rate. Once perfusion is resumed with serum enriched growth medium at 37°C, the metabolic rate of the cells recovers. This recovery period, called phase 1, could be distinguished by the increase in ³¹P-ATP (D of Fig. 3) and phosphocholine (PC) (B of Fig. 3) signal intensities. This process may take one or two days for our experience. In phase 2, however, the spectrum is marked by the intensities of ³¹P-ATP and PC staying constant, while the glycerophosphocholine (GPC) signal intensity continues to increase (C of Fig. 3). This suggests that the MCF-7 cells are in metabolic equilibrium, so all the ³¹P metabolites maintain steady signal intensity. Phase 3 is the best stage to conduct drug treatment experiments. It continues until the cells gradually die and all the metabolites decay. This process may last a few days.

Application 2: After establishing the baseline characteristics of MCF-7 cells in the improved agarose-enclosed cell perfusion system, we set out to determine the respiratory conditions of the cells under this improved system. The MCF-7 cells were perfused with medium containing either 0.1 mM lodoacetamide (a protein alkylating agent) or 10 mM Pentobarbital (a mitochondrial poison). The behavior of the ³¹P metabolites was quite interesting (see fig. 4, 5, and 6). For example, when the cells were perfused with lodoacetamide, there was an initial increase in the signals of PE, PC, GPC, and 2-ATP. The reason for this is not understood. Following the initial increase in signal intensities of these metabolites (see fig. 4E), there was a dramatic decline in signal intensities of these metabolites decaying the fastest (see fig. 4E), suggesting that lodoacetamide affected the proteins involved in ATP production pathways. The signal of inorganic phosphate (Pi) in these cells rose as the ATP concentration decreased. This was the case until the cells died. Cell death was supported from the fact that after treatment with lodoacetamide, the intracellular Pi signal gradually became extracellular Pi (see Fig. 5C) pay attention to the change in the chemical shift of Pi) suggesting either cell lysis or cell membrane leakage.

Even though Pentobarbital did not kill the cells, it had a similar effect on ³¹P metabolites as did lodoacetamide (see Fig. 6). All the ³¹P metabolites (except Pi) decreased in signal intensity after perfusion with Pentobarbital. Both of the drugs used in this experiment inhibit the enzymes critical to aerobic respiration by different pathways. The 2-ATP signal decayed the fastest (see fig. 6E) aerobic respiration or energy production. More experiments will have to be conducted to further support this argument.

Fig. 4. The spectra of MCF-7 cells perfused with complete medium containing 0.1 mM lodoacetamide. The perfusion time was 23 hours. The stacked spectra (A, B, C, D, and E) are the MCF-7 cells' ³¹P signals for phosphocholine and phosphocholine (PE and PC), inorganic phosphate (Pi), glycerophosphocholine (GPC) and diacylglycerol (DGE and GPC) and ³¹P-ATP. The stacked spectra (F, G, H, and I) are the MCF-7 cells' ³¹P signals for phosphocholine and phosphocholine (PE and PC), inorganic phosphate (Pi), glycerophosphocholine (GPC) and diacylglycerol (DGE and GPC) and ³¹P-ATP. The stacked spectra (A, B, C, D, and E) are the MCF-7 cells' ³¹P signals for phosphocholine and phosphocholine (PE and PC), inorganic phosphate (Pi), glycerophosphocholine (GPC) and diacylglycerol (DGE and GPC) and ³¹P-ATP. 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EARLY EEG & DIFFUSION MRI (dMRI) CHANGES IN AN EXPERIMENTAL MODEL OF SEVERE PERIVENTRICULAR LEUKOMALACIA (PVL)

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OBJECTIVES

The incidence of severe PVL in preterm neonates is 8 to 15 %. The poor neuro-developmental outcome, especially spastic diplegia occurs in majority of these infants. In addition, impaired visual and cognitive functions are also significant morbidity. While the etiology of PVL is multifactorial, cerebral ischemia (clinically silent) plays a dominant role. Therefore, early identification of ischemia is important for timely therapeutic intervention. The objective of the current study is to monitor EEG and dMRI profiles in the early hours of cerebral ischemia in an experimental PVL model.

DESIGN/METHODS

- Under ketamine-acetpromazine (KAY) isoflurane anesthesia, and strict aseptic technique, 15 mongrel dogs (1-3 wk old) underwent either sham operations without ischemia (SOC, N=4) or permanent bilateral common, external and internal carotid arteries occlusion (ISCH, N=11).
- Rectal temperature and transcutaneous O_2 saturation (O_2 sat) were monitored and during surgery and MRI measurements. The temperature was kept at 37-38°C, and the O_2 sat was maintained > 95 %.
- Flecillin 100,000 units/kg IM qd was given for 5 days, started 1 day prior to surgery.

RESULTS

- Fluid and nutrition:
 - RL 150cc/kg IV & SC
 - Gavage fed until it was returned to its dam.
 - Weaned to puppy's chow after a month of age
 - Body weight was recorded periodically
- EEGs were obtained via four scalp electrodes (Grass) placed in the extreme frontal and occipital regions of the head. The EEGs were recorded on paper and simultaneously into a digital computer programmed to read the frequency and duration of all defined waves. These data were compiled in a usable form by the computer in a time span of 10 seconds. This produces on-line frequency distributions as well as statistical and other displays. EEGs were recorded prior to, and within 6 h, 2 and 7 days after surgery.
- The animal was pre-sedated with KA and chloral hydrate or lorazepam for the dMRI study. A 4.7T, 33 cm, horizontal bore Varian MRI machine was used.
- Serial diffusion-weighted images were obtained prior, and within 7 h of surgery, and then repeated within 12 wk after surgery. The diffusion-weighted MRI technique was a modified spin-echo NMR imaging technique with two identical diffusion gradients were added before and after the 180° RF pulse. Four diffusion gradients ranging from 0 to 9 Gaus/cm in the x, y, and z directions were used to generate a series of diffusion weighted images in Figure 2). The apparent diffusion coefficients (ADC) were derived from these images shown in Figure 3. The Regions of interest (ROIs) were selected from the white matter in centrum semiovale, internal capsule, and optic radiation, in predetermined coronal MRI slices.
- Dogs were sacrificed at 3 months of age.
- Statistical analysis - One-way Anova analysis of variance per JMP program.
- Alpha = 0.05.

RESULTS

All 4 SOC survived, but, 7 out of 11 ISCH dogs died (5 at < 1 wk, and 2 at 3 & 4 wk postischemia). The EEG data were integrated over 1 to 14 cycles per second frequency band (CPSF). Compared to normal dogs prior to surgery (NSOC, N=17), there was a significant increase in ≤ 3 CPSFB in EEG within 5h postischemia in both SOC (N=4) and ISCH (N=9). However, it was more marked in ISCH (Fig 4 A-C, $P \leq 0.05$). In addition, ISCH and SOC had respectively a significant decrease in 8-14, and 11-14 CPSFB. However, the EEG of SOC and ISCH dogs normalized towards NSOC values 1 wk after surgery.

The ADC from the selected white matter areas were measured in SOC and ISCH dogs. The values of SOC were 0.065 to 0.121 mm²/sec. For the normal, non-involved areas of ISCH, the ADC values were between 0.066 to 0.085 mm²/sec. On Day 7 and Day 9 the ADC of the ischemic regions were 0.111 and 0.131 mm²/sec. The relative ADC value which was the ratio of ADC from the ischemic region to the non-involved opposite side of the brain were plotted as a function of time (Figure 5).

CONCLUSION

- Significant abnormal EEG patterns were observed within 6 hours of ischemia or sham-operation, but, the ISCH experienced marked increase in 1 to 3 CPSFB than the SOC dogs. Normalization of the EEG occurred 1 week after surgery in both groups. Anesthesia and sedation had profound effects on EEG.
- The relative ADC values decreased at 24 h of ischemia in the periventricular white matter (centrum semiovale), but, increased to above SOC values on ≥ 7 days of ischemia.
- All ISCH dogs revealed abnormal triphenyltetrazolium chloride stain in the PV white matter and thalamus 6 h after ischemia, and were subsequently associated with cystic PVL and motor deficits. SOC were normal.

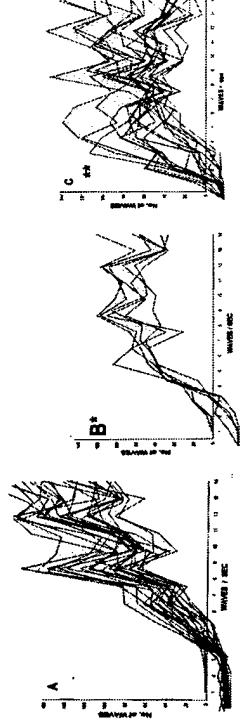


Figure 4. Frequency distribution patterns of EEG from NSOC (A), SOC (B), and ISCH (C). *p ≤ 0.05 (A compared with B), **p ≤ 0.05 (C compared with A or B) at 1-3 CPSFB.

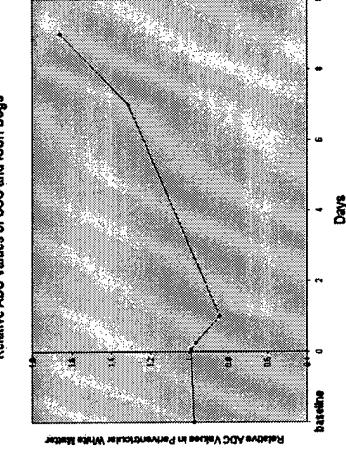


Figure 5 – Relative ADC values as a function of time. The relative ADC value is the ratio of the ischemic region to the non-involved area on the opposite side of the brain. The relative ADC initially dropped on the first day, however, it recovers and increases in comparison to the normal sham control.

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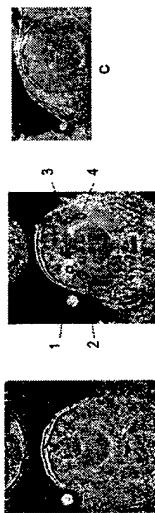


Figure 2 – Diffusion-weighted images for ISCH dog Day 7, with varying gradient strengths (0-3.57 gauss/cm). Ischemic area on the left side becomes darker as gradient strength increases.

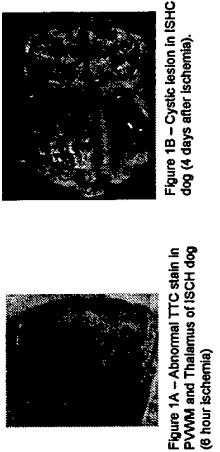


Figure 3 – Diffusion map images of derived in diffusion weighted images. Figure 3A shows an ISCH dog on the day of surgery. Figure 3B shows a second ISCH dog on day 7 after the surgery. The numbers 1, 2, 3, and 4 represent ROI's used to extrapolate the ADC values. Figure 3C shows an ISCH dog which is 3 months old.